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13. ABSTRACT (Maximum 200 Words) Prolactin (PRL) is a 23 kDa hormone that targets the breast, but its role in breast cancer is controversial. An N-terminal 16K PRL fragment suppresses endothelial cell proliferation, but its ability to inhibit tumor growth had not been tested. Our hypotheses were: 1) 16K PRL inhibits tumor growth by suppressing angiogenesis and 2) locally-produced 23K PRL promotes breast cancer growth. Recombinant 16K PRL failed to suppress the proliferation of endothelial cells unless contaminated with endotoxins. Overexpressing 16K PRL in MDA-MB-435 (MDA) breast cancer cells did not inhibit tumor growth in nude mice, but tumors from endostatin-overexpressing clones were 4-5 fold smaller than control tumors. These data confirm the potent anti-angiogenic activity of endostatin but do not support a similar function for 16K hPRL. Exogenous and overexpressed 23K PRL increased MDA-MB-435 cell proliferation and PRL-R expression <i>in vitro</i> . Tumors from 23K PRL-overexpressing clones grew 2-4 times faster than vector or wild type MDA tumors in nude mice and expressed higher levels of the PRL-R and the anti-apoptotic protein bcl-2 than vector controls. These data support a role for 23K breast PRL as a mitogenic/anti-apoptotic factor and suggest it may serve as a new therapeutic target for treating breast cancer.				
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Introduction

Tumors must induce the formation of new blood vessels in order to grow and metastasize. Active angiogenesis results from a disruption in the balance between angiogenic and angiostatic factors produced by the tumor. In breast cancer, a high density of blood vessels is inversely correlated with patient survival. Suppressing tumor growth by targeting its vasculature thus offers a promising therapeutic strategy. Prolactin (PRL) is a 23 kDa pituitary hormone that has mitogenic, morphogenic, and lactogenic actions on the breast. The role of 23K PRL in breast cancer is controversial, but its N-terminal 16K fragment suppresses proliferation of endothelial cells from several species, inhibits capillary formation in chick embryos, and antagonizes the actions of angiogenic factors. However, the ability of 16K PRL to inhibit tumors *in vivo* has not been tested. The purpose of this thesis work was to test the following hypotheses: 1) 16K PRL suppresses angiogenesis and tumor growth *in vivo* and 2) locally-produced 23K PRL promotes breast cancer growth. If our hypotheses are correct, treating breast tumors with 16K PRL should inhibit tumor vascularization and subsequent growth. In contrast, 23K PRL should increase breast cancer cell proliferation and tumor growth.

Training

I received a travel award for an oral presentation at the Gordon Research Conference on prolactin and presented a poster at the 84th Annual Meeting of the Endocrine Society. I also gave oral and poster presentations of my research at the University of Cincinnati for the Cell Biology Student Retreat and the Graduate Student Research Forum. I also presented my data at the Annual Symposium of Ryan Fellows as part of my Ryan fellowship, which is awarded to select students at the University of Cincinnati, Dartmouth, and Harvard. Other training included attending weekly seminars sponsored by the Department of Cell Biology and critiquing papers and discussing ongoing research projects in Dr. Ben-Jonathan's laboratory meetings. This research and training culminated in the successful preparation and oral defense of my thesis entitled "Prolactin as a local growth factor in breast cancer," and I was awarded a Ph.D. degree in June, 2002.

Research Objectives and Accomplishments

In the first two annual reports, the following accomplishments were described: 1) generation and characterization of polyclonal antibodies against prolactin (PRL), 2) development of a rapid dot blot assay to detect PRL, 3) production of recombinant 16K and 23K PRL, 4) establishment of an *in vivo* tumor model, 5) generation and characterization of stable MDA-MB-435 (MDA) clones over-expressing 16K or 23K human PRL (hPRL) or endostatin (as a control), and 6) optimization of protocols for immunohistochemical staining for PRL and von Willebrand factor in tissue sections.

Objective: To confirm the angiostatic activity of 16K PRL *in vitro*

Previous annual reports described technical difficulties with purifying recombinant 16K human PRL (16K rhPRL) and our concerns that endotoxin contamination might explain the angiostatic effect of 16K PRL on endothelial cells. As shown in Fig. 1, our first heparin-purified 16K rhPRL sample inhibited the proliferation of bovine aortic endothelial cells (BAEC) in a dose-dependent manner but had no effect on fibroblasts (Fig. 1B) or MDA-MB-435 breast cancer cells (Fig. 1C). 23K rhPRL had no effect on any cell type, and its activity did not differ from affinity-purified NIH pituitary hPRL.

However, Western blotting (Fig. 2A insert) showed little 16K rhPRL in the sample with angiostatic activity. Moreover, the angiostatic 16K rhPRL sample tested positive for endotoxins (data not shown), lipopolysaccharides from the cell wall of gram-negative bacteria that induce apoptosis in endothelial cells (1-2). Syringe-filtering the contaminated 16K rhPRL eliminated all of the 16K rhPRL from the sample but did not reduce its angiostatic activity (Fig. 2B). In contrast, none of the other endotoxin-negative, heparin-purified 16K rhPRL samples tested inhibited endothelial cell proliferation (Fig. 2C). Because 16K and 23K rhPRL were secreted into the insect cell media at very high concentrations (15-20 $\mu\text{g/ml}$), we also treated endothelial cells with unpurified or spin filtered rhPRL media, but no angiostatic activity was observed in these samples. To confirm that the BAEC cells responded to angiostatic compounds, they were incubated with different concentrations of TNP-470. TNP-470, a synthetic derivative of the fumagillin antibiotic, is a potent inhibitor of angiogenesis (3) and inhibited the proliferation of BAEC cells in a dose-dependent manner (data not shown). Recombinant endostatin was not commercially available when the BAEC proliferation assays were used to test the angiostatic activity of 16K rhPRL.

To determine whether 16K hPRL inhibits angiogenesis and tumor growth *in vivo*, MDA-MB-435 (MDA) cells were transfected with vectors encoding 16K hPRL, an empty vector, or endostatin as a control. Puromycin-resistant cells were cloned by limiting dilution and expanded. As shown by RT-PCR, 16K hPRL mRNA is expressed by two clones (16K1 & 16K2) but not by the vector control clones (V1 & V2). Two endostatin clones (E1 & E2) express endostatin mRNA (Fig. 3), but no endostatin expression was observed in the V1, V2, or 16K1 clones. The dot blot assay was then used to verify 16K hPRL and endostatin secretion by the clones (data not shown).

Before injecting the MDA clones into nude mice, we compared the growth rates of the various clones. As shown in Fig. 4A, the final cell number for both 16K hPRL clones was significantly ($P < 0.05$) lower than the V2 or E1 clones, suggesting that 16K hPRL can act as an antagonist to breast cancer cells. This finding was unexpected since angiostatic activity is specific for endothelial cells and 16K PRL has not been reported to have antagonist activities in other cell types. There was no difference in the *in vitro* growth rates of the vector (V1 & V2) and endostatin (E1 & E2) clones (Fig. 4B).

Objective: To test the angiostatic activity of 16K PRL and endostatin *in vivo*

To compare the effects of 16K hPRL and endostatin on tumor growth, MDA clones were inoculated into nude mice. All of the male mice injected with an endostatin-overexpressing clone (E1) developed tumors, but the tumors remained the same size for eight weeks (Fig. 5). In contrast, the tumors from the vector control clone (V2) continued to grow and were significantly larger ($P < 0.05$) than the endostatin tumors by week 5. Despite their slower *in vitro* growth rate, the 16K1 hPRL tumors grew significantly faster ($P < 0.05$) than both the vector and endostatin tumors, and these tumors had to be harvested early because of total tumor burden. Interestingly, the 16K hPRL tumors showed no signs of necrosis at week 7 despite their large size, and as evident by the picture, large blood vessels supplied the tumor. The tumors pictured (Fig. 5 insert) are representative of the various treatment groups at week 7, and Western blotting confirmed endostatin expression in tumor extracts from the E1 clones but not from the V2 clones.

When injected into the mammary fatpad of female nude mice, (Fig. 6), two different endostatin clones (E1 & E2) formed tumors that remained dormant for 10 weeks, while tumors from two vector control clones (V1 & V2) grew significantly ($P < 0.05$) larger than both endostatin tumor groups. Two 16K hPRL clones were also injected into female nude mice. The 16K1 tumors grew as rapidly as in the males and were significantly ($P < 0.05$) larger than both the vector and endostatin tumor groups by week 3 (data not shown). However, the number of tumors that

developed from the second 16K PRL clone (16K2) was not high enough to allow the analysis of tumor growth. Pictures (Fig. 6 insert) of two tumors per group were taken at week 8. Western blotting demonstrated endostatin expression by the endostatin tumors.

Summary and discussion regarding 16K PRL as an anti-angiogenic compound

In contrast to previous work showing that 16K recombinant hPRL inhibited endothelial cell proliferation in a dose-dependent manner (4), our heparin-purified 16K rhPRL was not angiostatic *in vitro* (Fig. 2). Several possibilities may account for the discrepancies between our data and previous reports. First, earlier studies used bovine brain or adrenal microvascular endothelial cells (4-5) rather than bovine aortic endothelial cells. Since the aorta must accommodate the high blood volume and pressure required to pump blood throughout the body, the endothelial cells lining the aorta and other large blood vessels may differ from those lining the smaller blood vessels of the brain and adrenal cortex. However, angiostatin inhibits the proliferation of bovine capillary and bovine aortic endothelial cells *in vitro* without affecting the proliferation of carcinoma cells, fibroblasts, or smooth muscle cells (6). Moreover, TNP-470 effectively inhibited the proliferation of BAEC cells at similar doses to those used to inhibit the proliferation of human umbilical vein endothelial cells (HUVEC) or to prevent endothelial tube formation (7-8). The only 16K rhPRL sample that inhibited BAEC proliferation was contaminated with high levels of endotoxins. This finding raises the possibility that the reported angiostatic activity of 16K PRL (4-5) may be the result of endotoxin contamination. Endothelial cells are especially sensitive to endotoxins target (9), while epithelial cells are less sensitive (10). When MDA clones overexpressing 16K hPRL were injected into nude mice, the 16K1 tumors grew faster than both the vector and endostatin tumors and were highly vascularized. Tumor growth rates were not obtained from the 16K2 clone because many of these mice did not develop tumors at all. Because of clonal variation, we cannot reach any definitive conclusions about the ability of 16K hPRL to inhibit angiogenesis and tumor growth in MDA breast cancer cells *in vivo* without repeating these experiments with additional 16K hPRL clones.

The MDA clones overexpressing endostatin effectively inhibited tumor growth in both male and female nude mice. These data support previous studies showing that endostatin is a potent angiostatic agent (11). Although recombinant endostatin proteins (11-13) as well as adenoviruses (14) and plasmids (15) encoding endostatin have been successfully used to inhibit the growth of a variety of tumors, this is the first report on the generation of stable clones overexpressing endostatin.

In summary, recombinant 16K hPRL was difficult to purify and did not inhibit the proliferation of endothelial cells *in vitro*. However, TNP-470 effectively repressed the growth of BAEC cells. No evidence for reduced tumor growth was observed when MDA clones overexpressing 16K hPRL were injected into nude mice, although our conclusions are limited to observations of a single clone. In contrast, endostatin overexpression in MDA breast cancer cells potently suppressed tumor growth by inhibiting angiogenesis. Because of its hydrophobic nature and questionable angiostatic activity, we believe 16K PRL has little potential as a therapeutic compound. As a further consequence of these negative results, we did not attempt to characterize 16K PRL receptors in breast tissue.

Objective: To determine whether 23K hPRL accelerates tumor growth in nude mice

The role of 23K PRL in breast cancer has been controversial. In rodents, PRL promotes both spontaneous and carcinogen-induced mammary tumors. In humans, however, drugs that suppress circulating PRL do not improve patient survival, and the correlation between serum PRL levels and breast cancer risk is weak. However, PRL is mitogenic in breast cancer cells, and both PRL

and its receptors (PRL-R) are expressed by the majority of breast carcinomas, with higher PRL-R mRNA expression in tumors than in adjacent normal tissue. To determine whether locally-produced 23K PRL acts as an autocrine/paracrine growth factor in breast cancer, we first confirmed that wild type MDA cells express 23K PRL and PRL-R mRNA. We next showed that exogenous 23K PRL increased proliferation and PRL-R mRNA expression in wild-type MDA cells and that MDA clones overexpressing 23K PRL proliferated faster than vector control clones *in vitro* (previous report).

To determine the effects of locally-produced PRL on tumor growth, MDA clones overexpressing 23K PRL were inoculated into nude mice. The tumors that formed from three different PRL-overexpressing clones grew significantly ($P < 0.05$) larger than vector control tumors in female mice (Fig. 7). The clone secreting the highest amount of hPRL (23K2) grew faster than clones that secreted lower levels of hPRL. Analysis of tumor growth rates from weeks 4-7 showed 2-4 fold faster growth rates ($P < 0.05$) for the 23K hPRL tumors as compared to the vectors. We also examined the effect of hPRL overexpression on subcutaneous tumor growth in male nude mice. Fig. 8 shows that a hPRL overexpressing clone formed tumors that grew significantly ($P < 0.05$) larger than a vector control (V2) or wild type (WT) cells in a different hormonal background. The tumor photographs (Figs. 7 and 8) are representative of the various treatment groups at week 8.

To verify PRL production and examine PRL-R expression in the tumors, tumor extracts were analyzed by Western blotting. PRL was detected in all three 23K PRL clones but not in the vectors (Fig. 9). Expression of the PRL-R also was markedly higher in the 23K hPRL clones than in the vectors. Because PRL is a survival factor in Nb2 cells and increases bcl-2 expression (16), we compared the expression of the anti-apoptotic protein bcl-2 and the pro-apoptotic protein bax in the various tumors. Interestingly, bcl-2 expression increased in tumors overexpressing hPRL while bax levels were unchanged. Quantitation of the Western blots showed significantly ($P < 0.05$) higher PRL/ β -actin, PRL-R/ β -actin and bcl-2/bax ratios in 23K hPRL tumors than vectors.

The PRL-overexpressing tumors also had metastasized to the lymph nodes and were highly vascularized. PRL staining was detected in 23K2 tumor sections and in the lymph nodes of mice with PRL-overexpressing tumors by immunohistochemistry (Fig. 10), suggesting that PRL-producing cells had metastasized. In contrast, no staining was observed in the tumors or lymph nodes from the vector control (V2) or in those stained with normal rabbit serum (NRS). The number of metastases from mice injected with hPRL-overexpressing clones vs. controls was not analyzed. The 23K2 hPRL were highly vascularized (Fig. 11), as seen in sections stained with von Willebrand factor, a marker for endothelial cells used to assess blood vessel density in tumors. The V2 control tumors are also well vascularized, but no difference between the microvessel density in the PRL-overexpressing tumors and the controls was found.

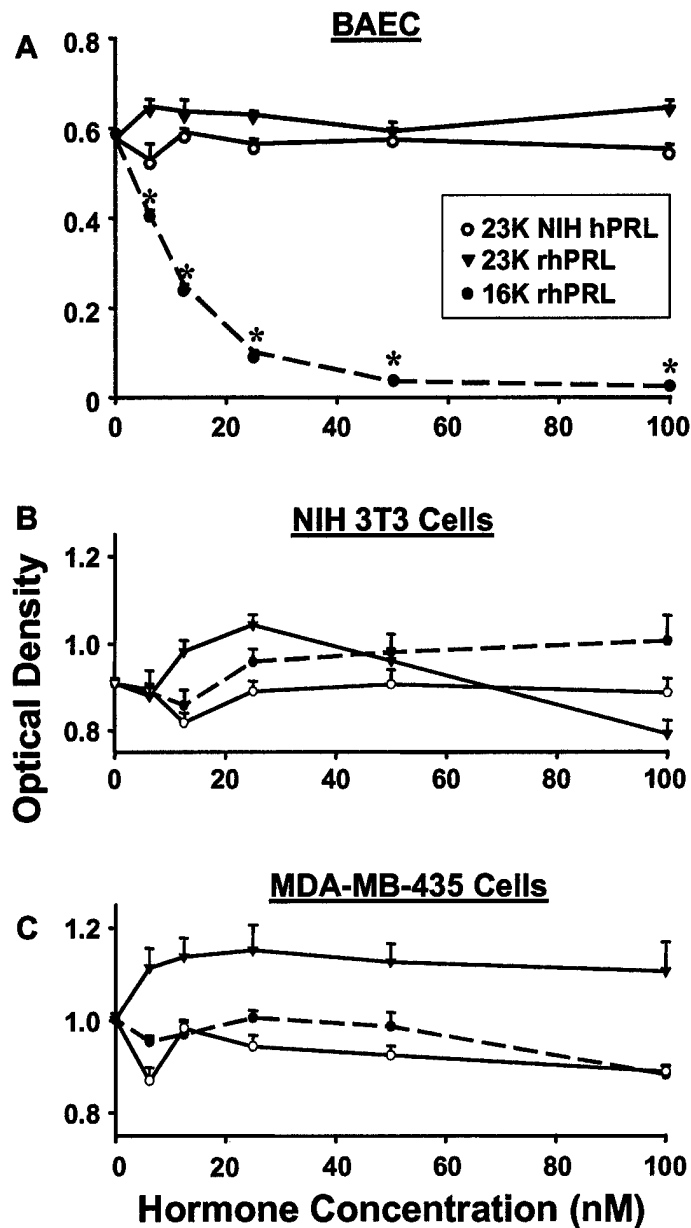


Fig 1. Inhibition of endothelial cell proliferation by heparin-purified 16K rhPRL. Recombinant PRLs purified by heparin affinity chromatography were quantified by the dot blot assay. Bovine aortic endothelial cells (BAEC), mouse fibroblasts (3T3), and human breast cancer cells (MDA-MB-435) were incubated with different concentrations of 16K or 23K rhPRL. After 3 days, cell number was determined by the MTT assay. Note the dose-dependent inhibition of BAEC cells by 16K rhPRL. The inhibitory action of 16K rhPRL was specific for endothelial cells, and 23K rhPRL had no effect on any of the cells. *, $P < 0.05$ (vs. both 23K NIH and rhPRL).

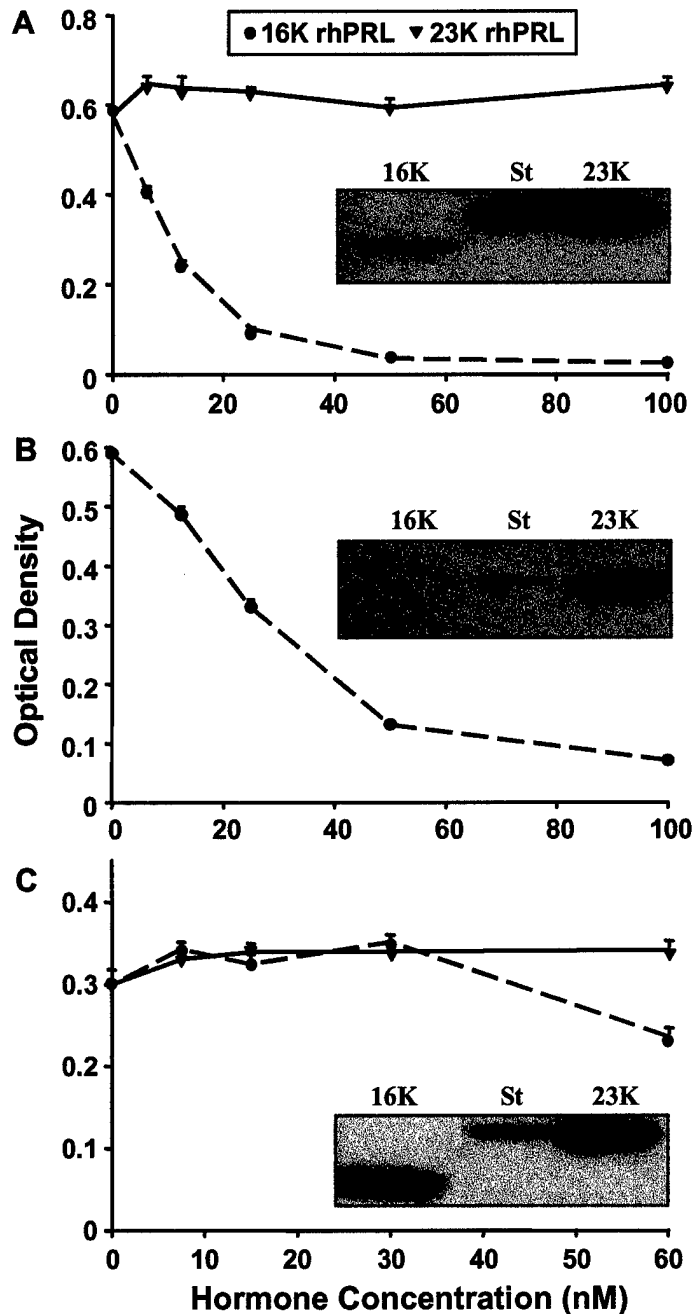


Fig 2. Other 16K rhPRL samples do not inhibit endothelial cell proliferation. Heparin-purified rhPRL was analyzed by the dot blot assay and Western blotting. BAEC cells were treated with different concentrations of 16K or 23K rhPRL and cell number determined by the MTT assay. (A) Western blotting (insert) of the sample with angiostatic activity shows little 16K rhPRL. (B) Syringe-filtering the angiostatic sample eliminated the 16K rhPRL, but the sample retained its activity. (C) Other 16K rhPRL samples did not inhibit endothelial cell proliferation. St = 23K NIH hPRL.

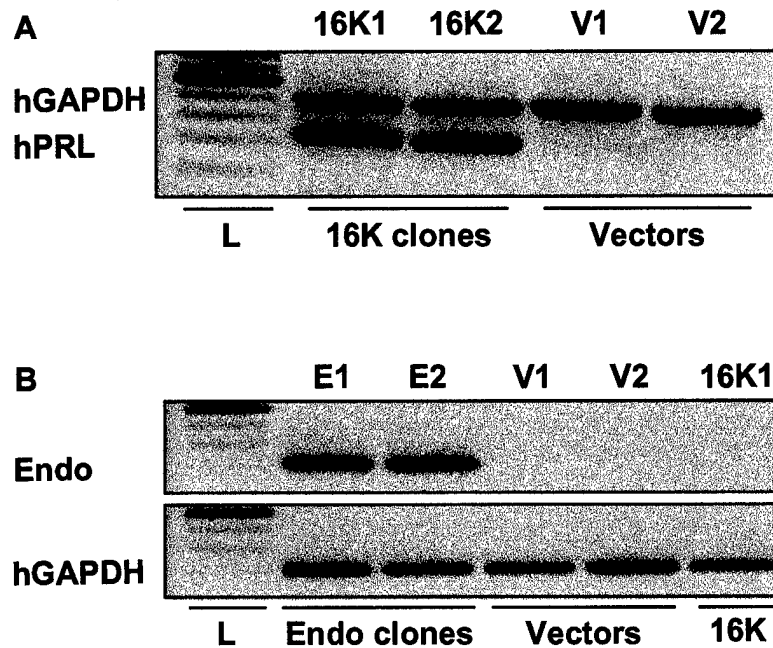


Fig 3. 16K hPRL and endostatin mRNA expression in stable MDA clones. Various clones were screened for 16K hPRL (A) or endostatin (B) mRNA expression by RT-PCR. GAPDH was used as a control. Expected product sizes are 328 bp for 16K hPRL and 500 bp for hGAPDH (A) and 255 bp for endostatin and 309 bp for hGAPDH (B). L = 100 bp ladder.

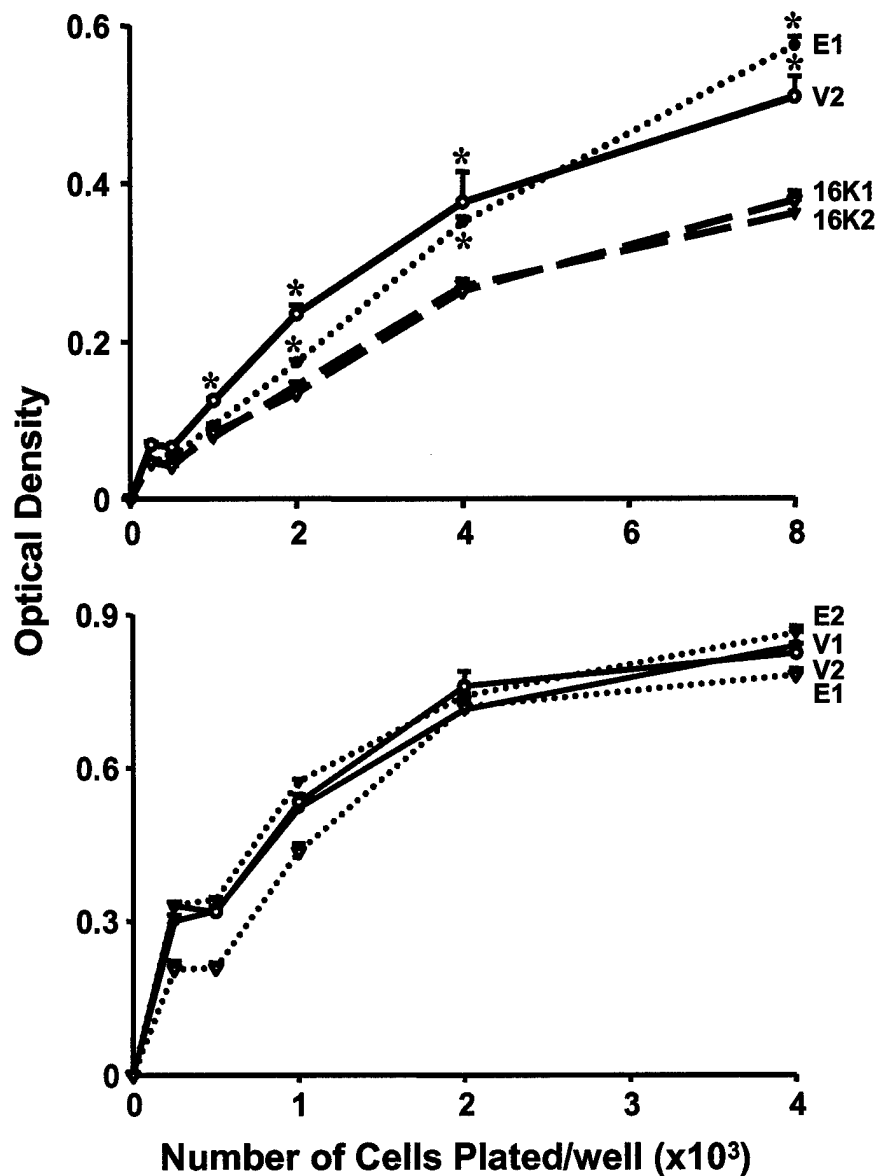


Fig 4. Growth rates of MDA clones overexpressing endostatin or 16K hPRL and vector controls. MDA clones were serially diluted from an initial concentration of 8000 cells/well and cultured in 5% FBS. Cell number was determined after 3 days by the MTT assay. Each value is a mean \pm SEM of 4 replicates, and the two graphs are from two different experiments. *, $P < 0.05$ (vs. both 16K hPRL clones).

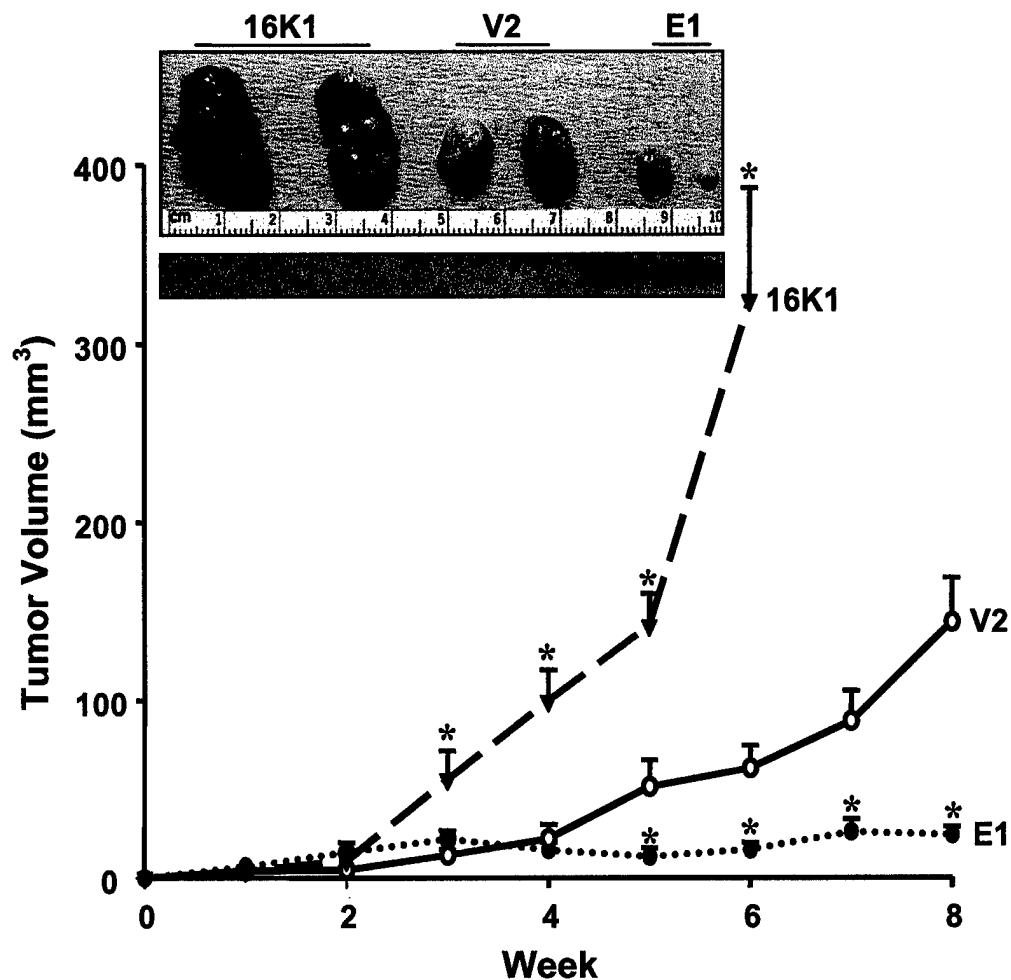


Fig 5. Endostatin-overexpression inhibits tumor growth, but 16K hPRL-overexpression accelerates tumor growth in male nude mice. MDA cells were injected subcutaneously into male nude mice. Each value is a mean \pm SEM of 6-8 mice/group. *, $P < 0.05$ vs. vector. Two representative tumors from each treatment group at week 7 are shown. Western blotting shows endostatin expression in tumor extracts from the endostatin-overexpressing clones.

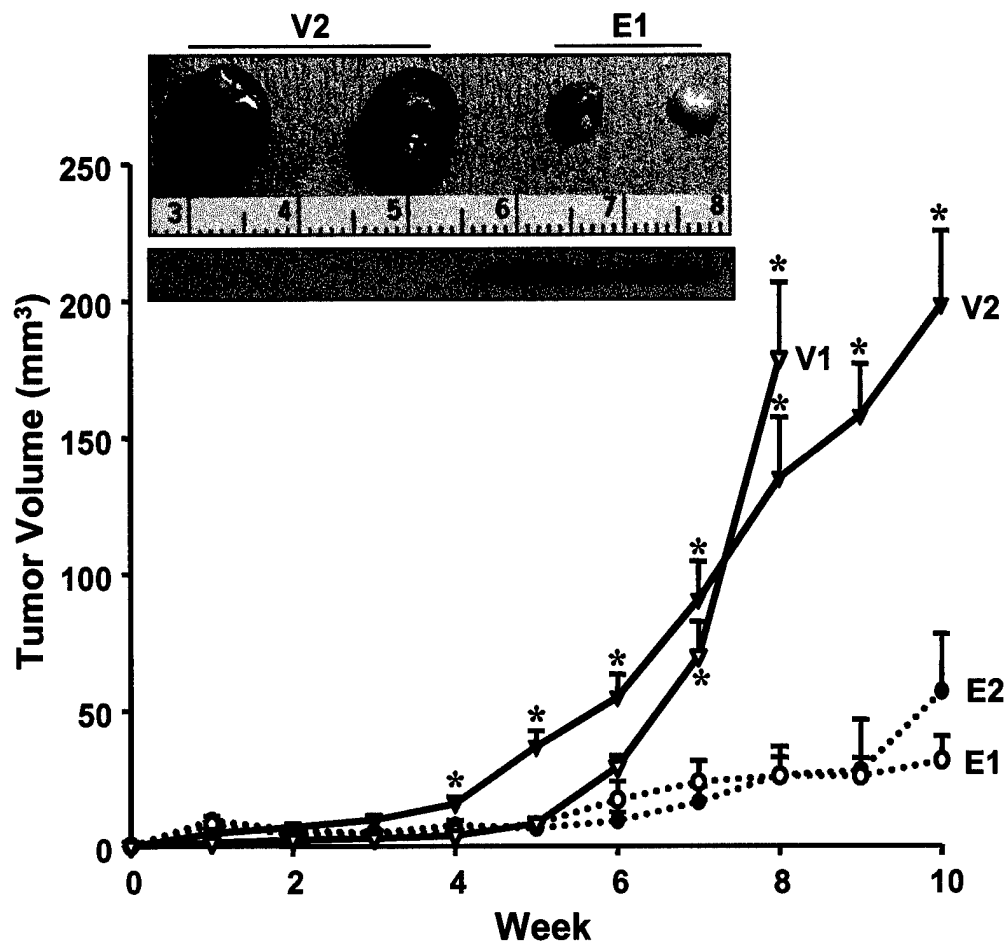


Fig 6. Endostatin-overexpression suppresses tumor growth in female nude mice. MDA cells were injected into the mammary fatpad of female nude mice. Each value is a mean \pm SEM of 8-10 mice/group. *, $P < 0.05$ vs. both endostatin clones. Representative tumors from the treatment groups are shown at week 8. Endostatin expression in tumors extracts is shown by Western blotting of the endostatin-overexpressing clones.

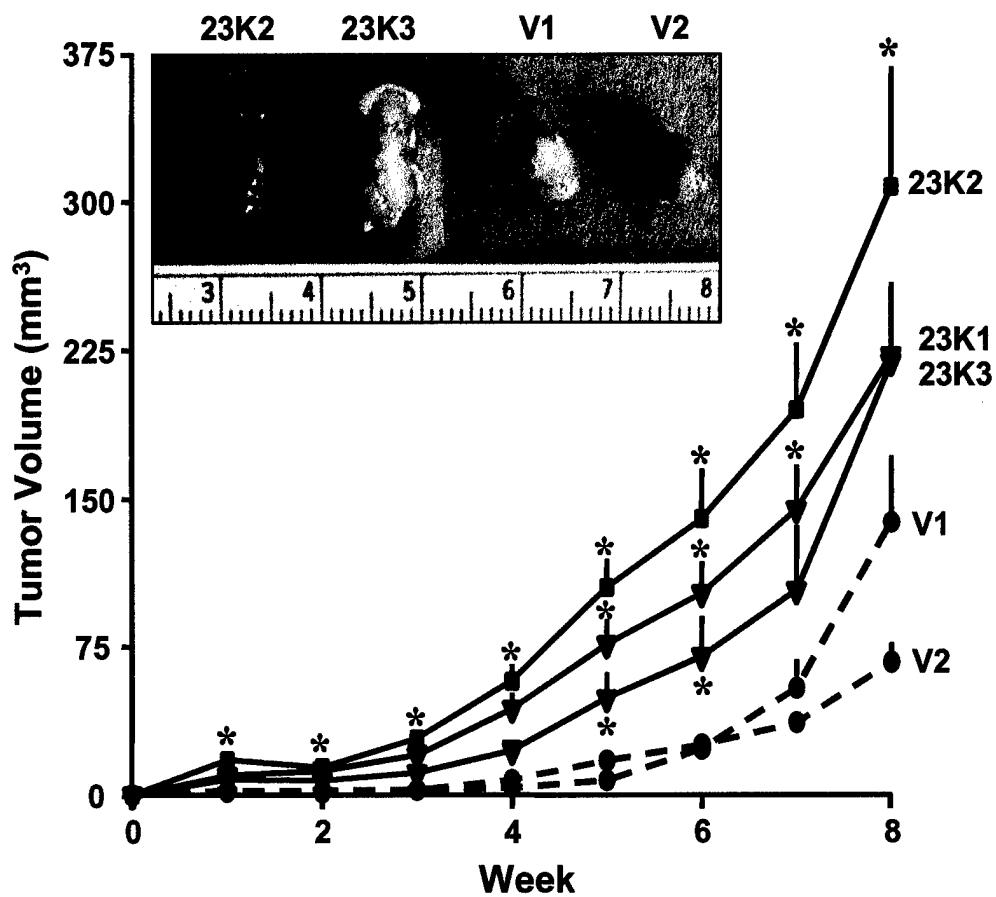


Fig. 7. PRL-overexpression accelerates tumor growth in female nude mice. MDA cells were injected into the mammary fatpad of female nude mice. Each value is a mean \pm SEM of 8-10 mice/group. *, $P < 0.05$ vs. both vectors. The photograph shows representative tumors from different treatment groups at week 8.

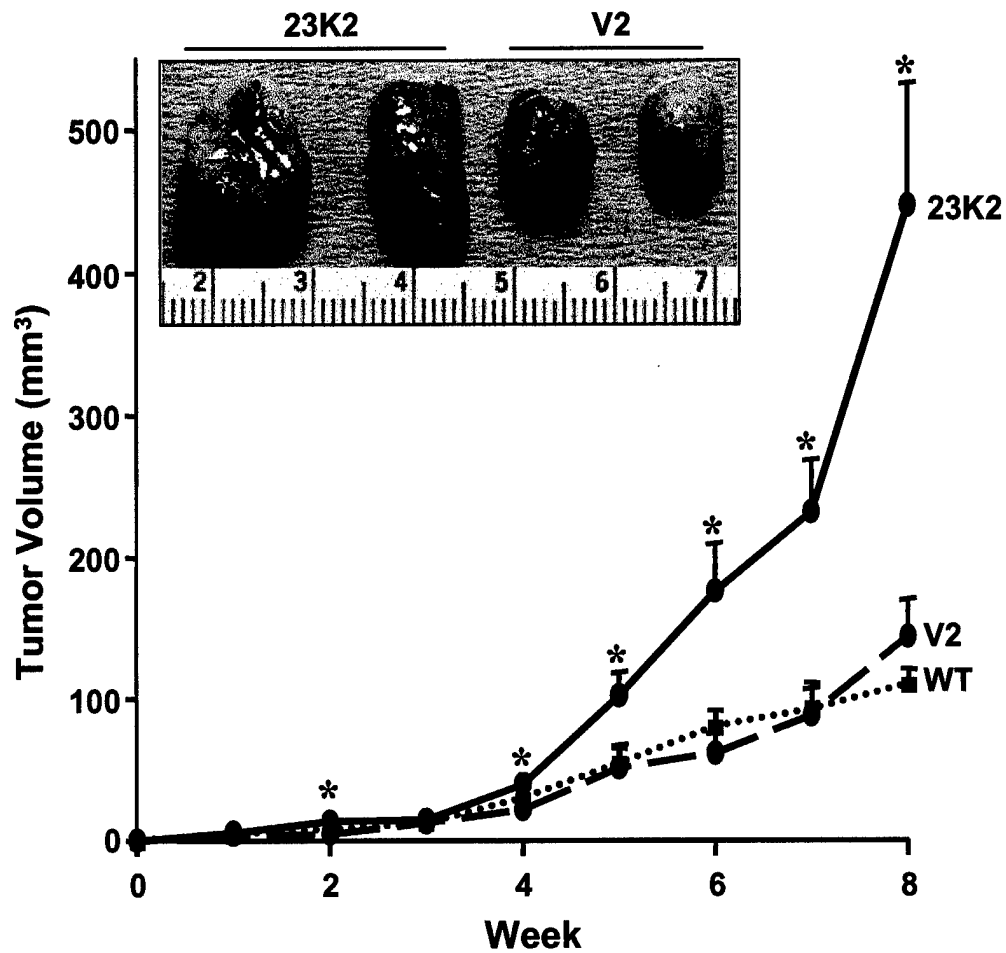


Fig. 8. PRL-overexpression accelerates tumor growth in male nude mice. MDA cells were injected subcutaneously in male athymic mice. Each value is a mean \pm SEM of 8-10 mice/group. *, $P < 0.05$ vs. vector. Two representative tumors from each treatment groups at week 8 are shown. WT = wild type.

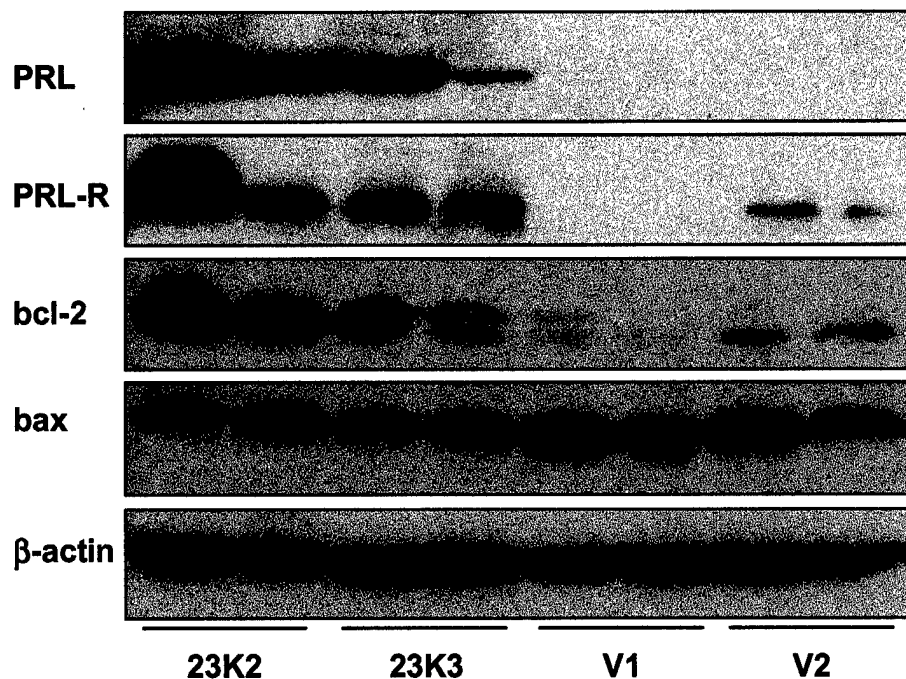


Fig. 9. PRL-R and bcl-2 proteins are increased in tumors overexpressing hPRL. Tumor lysates (150 μ g proteins) from the different MDA clones were separated by SDS-PAGE. Following transfer, the membranes were incubated with various antibodies. β -actin was used as a loading control.

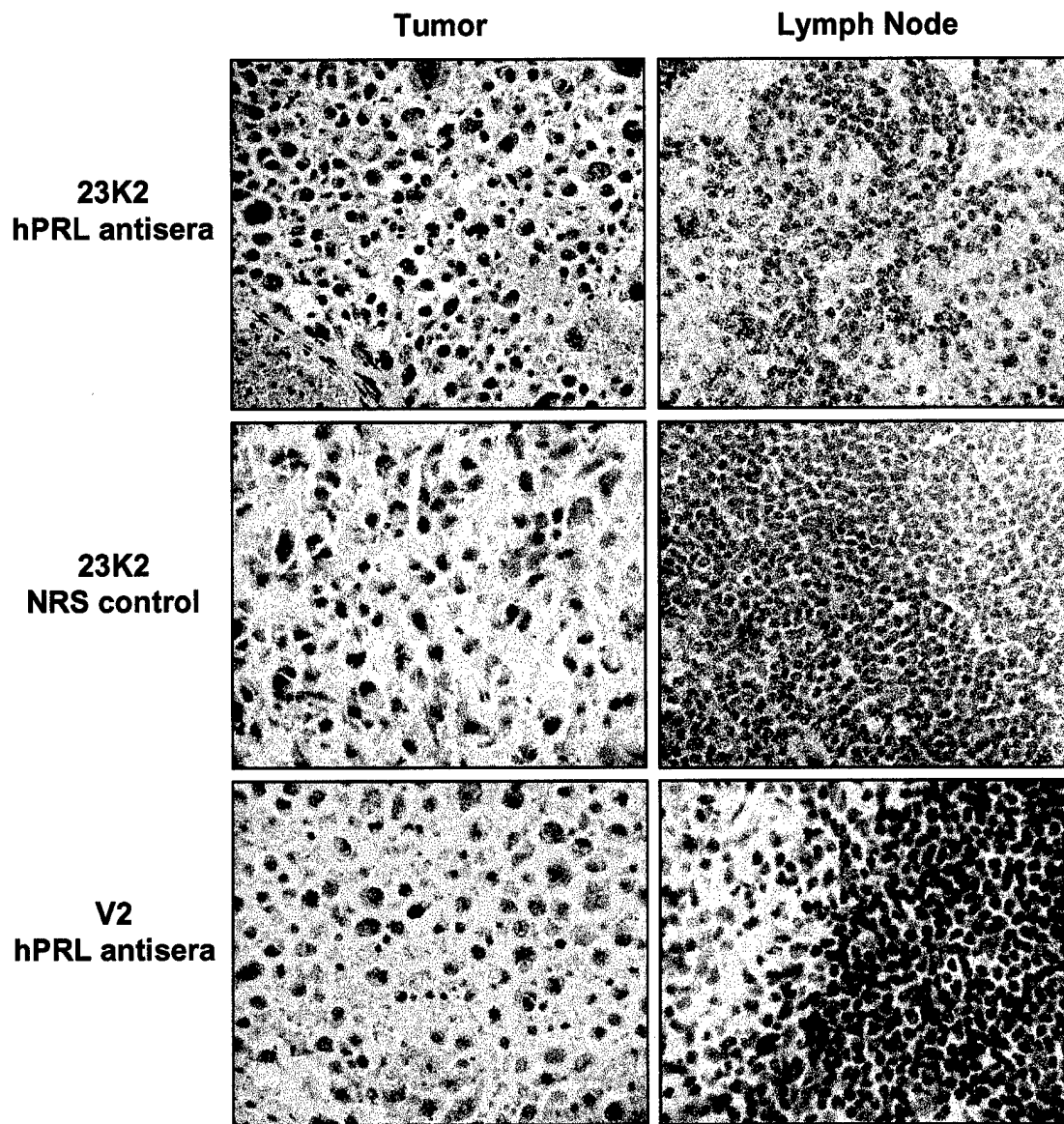


Fig. 10. Immunohistochemistry for PRL in tumors and lymph nodes. Sections from a hPRL-overexpressing tumor and vector control tumor were stained with hPRL antisera or normal rabbit serum (NRS) as a control (left panels). Lymph nodes from the same treatment groups were also stained with these antibodies (right panels). Magnification x40.

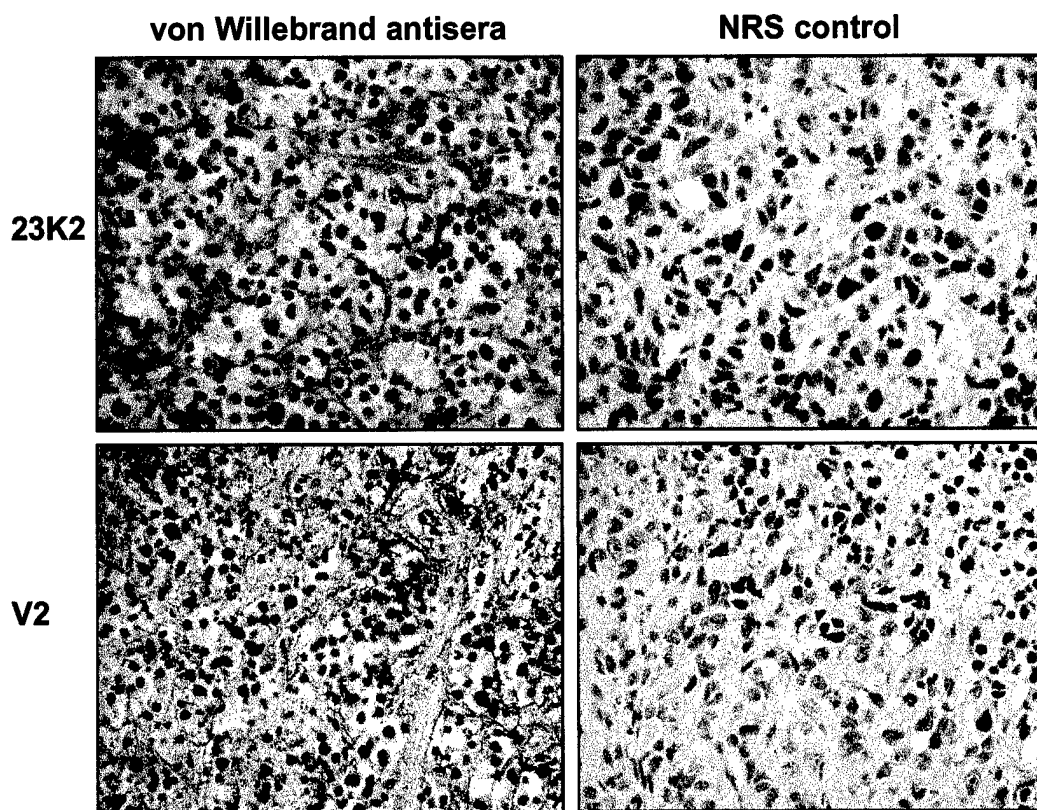


Fig. 11. Immunohistochemistry for von Willebrand factor in tumors. Sections from a hPRL-overexpressing and a vector control tumor were stained with antisera against von Willebrand factor to detect blood vessel density in the tumors. Magnification $\times 40$. NRS = normal rabbit serum control.

Key Research Accomplishments

- Recombinant 16K hPRL does not inhibit endothelial cell proliferation *in vitro* unless contaminated with endotoxins
- Preliminary studies suggest that MDA-MB-435 (MDA) clones overexpressing 16K PRL do not inhibit angiogenesis or tumor growth when injected into nude mice
- Endostatin overexpression in MDA cells suppresses tumor growth in nude mice by inhibiting angiogenesis
- Exogenous 23K hPRL increases MDA cell proliferation and PRL receptor (PRL-R) expression
- MDA clones overexpressing 23K hPRL proliferate faster and express higher levels of the PRL-R than vector control clones *in vitro*
- 23K PRL-overexpressing clones form tumors in nude mice that grow 2-4 times faster than tumors from vector control clones or wild type MDA cells
- Expression of the PRL-R and the anti-apoptotic protein bcl-2 are higher in tumors expressing 23K hPRL than in control tumors

Reportable Outcomes

Manuscripts

Liby K, Neltner B, Mohamet L, Menchen L, Ben-Jonathan N. Prolactin overexpression by human breast cancer cells accelerates tumor growth. Submitted to Cancer Research.

Liby K, Dean G, Ben-Jonathan N. Recombinant 16K human prolactin is not angiostatic *in vitro*. In preparation for submission to Cancer Letters.

Liby K, Nelner B, Mohamet L, Ben-Jonathan N. Overexpression of endostatin in MDA-MB-435 breast cancer clones inhibits tumor growth. In preparation for submission to Cancer Research.

Abstracts

Liby K, Neltner B, Mohamet L and Ben-Jonathan N. Endostatin overexpression in MDA-MB-435 breast cancer clones suppresses tumor growth by inhibiting angiogenesis. Poster presentation, The 84th Annual meeting of the Endocrine Society, San Francisco, CA; 2002.

Presentations

Liby K, Neltner B and Ben-Jonathan N. Prolactin as a local growth factor in MDA-MB-435 human breast cancer cells. Gordon Research Conference on Prolactin. Ventura, CA; 2002.

Liby K and Ben-Jonathan N. Prolactin as a local growth factor in breast cancer. Annual Symposium of Ryan Fellows. Dartmouth College. Holderness, NH; 2002.

Degree

Ph.D. – University of Cincinnati College of Medicine. Department of Cell and Molecular Biology. Thesis: 23K prolactin as a local growth factor in breast cancer. June, 2002.

Conclusions

I have generated recombinant 16K and 23K hPRL, but heparin-purified 16K hPRL failed to inhibit endothelial cell proliferation unless the samples were contaminated with endotoxins. MDA-MB-435 (MDA) clones that overexpress 16K PRL, 23K PRL, endostatin, or vector controls were generated, and PRL and endostatin production and secretion were verified. In preliminary experiments, MDA clones overexpressing 16K PRL did not inhibit angiogenesis or tumor growth in nude mice whereas tumors from the endostatin clones were significantly smaller than vector controls. These data confirm the potent anti-angiogenic and anti-tumorigenic activity of endostatin but do not support a similar function for 16K PRL.

The growth-promoting effects of 23K PRL were also examined. Exogenous hPRL significantly increased MDA cell proliferation and upregulated PRL-R mRNA expression in wild-type MDA cells. MDA clones overexpressing 23K PRL also proliferated faster and expressed higher levels of the PRL-R protein than vector control clones. When injected into female and male nude mice, the 23K PRL overexpressing clones formed tumors that grew 2-4 fold faster than control tumors. Western analysis demonstrated higher PRL levels, increased PRL-R protein expression, and upregulation of the anti-apoptotic protein bcl-2 in the PRL-overexpressing tumors. These data support a role for local PRL as a mitogenic/anti-apoptotic factor in human breast cancer and suggest that it may serve as a new therapeutic target for the treatment of breast cancer.

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Endostatin overexpression in MDA-MB-435 breast cancer clones suppresses tumor growth by inhibiting angiogenesis.

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Tumors must induce the formation of new blood vessels in order to grow and metastasize. Active angiogenesis results from a disruption in the balance between angiogenic and angiostatic factors produced by the tumor. In breast cancer, a high density of blood vessels is inversely correlated with patient survival. Endostatin, a cleaved product of collagen XVIII, inhibits endothelial cell proliferation and suppresses tumor growth and metastases. As our experimental model, we chose MDA-MB-435 (MDA) human breast cancer cells. These estrogen receptor-negative cells are tumorigenic and metastatic in nude mice. Our objectives were to: a) generate and characterize stable MDA clones overexpressing endostatin, b) compare tumor growth in nude mice inoculated with endostatin clones or vector controls, and c) examine the apoptotic index and blood vessel density in the tumors. MDA cells were transfected with an endostatin expression vector; controls were transfected with an empty vector. Endostatin production and secretion by the various clones were confirmed by RT-PCR and a dot blot assay. No differences were observed in the growth rates of the endostatin and vector control clones *in vitro*. Four different clones were injected into the mammary fatpad of female or subcutaneously in male nude mice. The volume of control tumors increased 10-15 fold from week 1 to 10. In contrast, endostatin tumors grew 2 fold from week 1 to 4 but then remained largely unchanged for the next 4-6 weeks. Similar results were observed in both male and female mice, and no differences were observed in the growth of vector control or wild type tumors. The apoptotic index was significantly higher in the endostatin tumors (5.6%) compared to vector control tumors (2.0%). Immunohistochemical staining for von Willebrand factor showed a marked reduction in blood vessel density in the endostatin tumors compared to controls. In conclusion, endostatin overexpression in MDA cells suppresses tumor growth by inhibiting angiogenesis and increasing apoptosis. (Supported by Army grant DAMD17-99-1-9128 and NCI CA80920).